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14. ABSTRACT This basic research initiative is geared ultimately toward developing effective and safe non-lethal technologies that alter skeletal muscle contraction and/or neural functioning via radiofrequency (RF)/microwave (MW) electromagnetic radiation. Major accomplishments included 1) near completion of studies examining the effect of 1 to 6 GHz MW fields on catecholamine release from chromaffin cells; 2) initiating studies using a novel exposure system for real-time imaging of intracellular effects in chromaffin cells in response to high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with the frequency spectrum centered in the band 0.75–6 GHz; 3) completion of studies on the effect of 0.75 to 1 GHz RF fields on skeletal muscle contraction using fixed frequencies and just recently implementing frequency sweep paradigms; 4) initiation of studies to examine the effect of nanosecond electric pulses of high intensity on catecholamine release from chromaffin cells. The research, which was presented at four international meetings and culminated in three peer-reviewed papers, has been transitioned into AFOSR grant FA9550-07-1-0592.					
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FINAL PERFORMANCE REPORT

Technical Proposal entitled: “Non-lethal weapons the use radiofrequency/microwave energy for stunning/immobilization”

Award Number: FA9550-06-1-0377

Start Date: 15 May 2006

Termination Date: 14 November 2008

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ABSTRACT

This basic research initiative is geared ultimately toward developing effective and safe non-lethal technologies that alter skeletal muscle contraction and/or neural functioning via radiofrequency (RF)/microwave (MW) electromagnetic radiation. Major accomplishments included 1) near completion of studies examining the effect of 1 to 6 GHz MW fields on catecholamine release from chromaffin cells; 2) initiating studies using a novel exposure system for real-time imaging of intracellular effects in chromaffin cells in response to high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with the frequency spectrum centered in the band 0.75–6 GHz; 3) completion of studies on the effect of 0.75 to 1 GHz RF fields on skeletal muscle contraction using fixed frequencies and just recently implementing frequency sweep paradigms; 4) initiation of studies to examine the effect of nanosecond electric pulses of high intensity on catecholamine release from chromaffin cells. The research, which was presented at four international meetings and culminated in three peer-reviewed papers, has been transitioned into AFOSR grant FA9550-07-1-0592.

EXECUTIVE SUMMARY

Objectives

The research in this proposal was to sustain the progress and growth of on-going research projects in which non-thermal radiofrequency /microwave (RF/MW) effects on skeletal muscle contraction and catecholamine release from chromaffin cells are being investigated. Another objective was to develop the capability to explore effects of nanosecond duration, high intensity electric field effects on chromaffin cells.

Accomplishments/New Findings

- 1) Effect of MW fields (1 to 6 GHz frequency range) on catecholamine release: studies are close to completion with apparent non-thermal effects on CA release noted most often with a novel delivery of 5-6 GHz MW fields.
- 2) Effect of RF/MW fields on intracellular calcium level: Experiments have recently gotten underway using a novel exposure system for real-time imaging of intracellular effects in chromaffin cells in response to high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with the frequency spectrum centered in the band 0.75–6 GHz.
- 3) Effect of RF fields (0.75 to 1 GHz frequency range) on skeletal muscle contraction: studies carried out using fixed frequencies have not shown any effects due to the RF fields; novel exposure protocols, similar to those alluded to in 1), are now being implemented.
- 4) Effect of high intensity nanosecond electric pulses on chromaffin cells: with pulsers provided by colleagues at the University of Southern California (USC), a single 4 nanosecond, 5 MV/m electric pulse was found to produce a pronounced, transient influx of calcium into the cells that is sufficient in magnitude to stimulate catecholamine release; no discernible adverse effects of the pulse have been observed.

Publications

- Yoon, J., Chatterjee, I., McPherson, D., and Craviso, G. L. (2006). Design, characterization and optimization of a broadband mini exposure chamber for studying catecholamine release from chromaffin cells exposed to microwave radiation: Finite-Difference Time-Domain Technique. *IEEE Transactions on Plasma Science*, 34, 1455-1469.
- Lambrecht, M., Chatterjee, I., McPherson, D., Quinn, J., Hagan, T., Craviso, G. L. (2006). Design, characterization and optimization of a waveguide-based RF/MW exposure system for studying nonthermal effects on skeletal muscle contraction. *IEEE Transactions on Plasma Science*, 34, 1470-1479.
- Vernier, P.T., Sun, Y., Chen, M.-T., Gundersen, M.A., and Craviso, G.L. (2008). Nanosecond electric pulse-induced calcium entry into chromaffin cells. *Bioelectrochemistry*, 73, 1-4.

Personnel Supported

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Interactions/Transitions

a) *Presentations*

Oral:

- Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G.L., Design and finite-difference time-domain characterization of a novel in vitro exposure device for real-time monitoring of changes in intracellular calcium due to pulsed RF/microwave electric fields. 28th Annual Bioelectromagnetics Society Meeting, Cancun, Mexico, June 2006.
- Craviso, G.L., Sun, Y., Chen, M-T, Gundersen, M.A. and Vernier, P.T. Single Nanosecond Electric Pulse Elevates Intracellular Calcium in Bovine Adrenal Chromaffin Cells. 28th Annual Bioelectromagnetics Society Meeting, Cancun, Mexico, June 2006.
- Craviso, G. L., Sun, Y., Chen, M., Gundersen, M. and Vernier, T., "Elevation of intracellular calcium in bovine adrenal chromaffin cells subjected to a single nanosecond electric pulse.", Gordon Conference on Bioelectrochemistry, Aussois, France. September 2006.
- Craviso, G.L., Vandenberg, P., Baird, S., Wiese, R., McPherson, D. and Chatterjee, I. Contractile force of mouse flexor digitorum brevis at supraphysiological temperatures. 29th Annual Bioelectromagnetics Society Meeting, Kanazawa, Japan. June 2007.
- Yoon, J., Chatterjee, I., McPherson, D. and Craviso, G.L.. Improvements to a free-space broadband in vitro microwave exposure system for on-line monitoring of catecholamine release from chromaffin cells. 29th Annual Bioelectromagnetics Society Meeting, Kanazawa, Japan. June 2007.
- Craviso, G. L., Maalouf, G., Choe, S., Chen, M. T., McPherson, D., Chatterjee, I., Gundersen, M. A. and Vernier, P. T. Nanosecond electric pulse stimulates catecholamine release from chromaffin cells. 30th Annual Bioelectromagnetics Society Meeting, San Diego, CA, June 2008.

Poster:

- Misra, R., Chatterjee, I., Yoon, J., McPherson, D. and Craviso, G.L., Thermal modeling of a free space exposure system for on-line monitoring of catecholamine release from chromaffin cells exposed to microwave fields. 29th Annual Bioelectromagnetics Society Meeting, Kanazawa, Japan. June 2007.
- Guan, W., Hagan, T., Chatterjee, I., McPherson, D. and Craviso, G. L., Narrowband and broadband radiofrequency/microwave exposure system for real-time monitoring of cellular responses. 30th Annual Bioelectromagnetics Society Meeting, San Diego, CA, June 2008.
- Craviso, G. L., Maalouf, G., Choe, S. McPherson, D. Chatterjee, I., Gundersen, M. A. and Vernier, P. T., Nanosecond electric pulse-induced catecholamine release from chromaffin cells. Gordon Conference on Bioelectrochemistry, Biddeford, ME, July 2008.

Other:

The research was featured in the June 2008 issue of Defense Technology International

b) Consultative and advisory functions – None

c) Transitions –The research project will transition into AFOSR FA9550-07-1-0592.

New Discoveries, Inventions or patent disclosures: None

Honors/Awards - None

COMPREHENSIVE TECHNICAL SUMMARY

Rationale

A plausible way to disrupt human functioning is to alter neuronal activity and skeletal muscle contraction. With this as our objective, we are investigating the feasibility of using RF/MW radiation to design non-lethal weapons that target these physiological processes. Special emphasis has been given to studying non-thermal RF/MW effects on two *in vitro* biological systems, neurosecretory adrenal chromaffin cells that synthesize, store and release catecholamines, and skeletal muscle that is responsible for voluntary movement. For carrying out these studies, a consideration of foremost importance was to design, fabricate, characterize and test RF/MW exposure systems that allow maximal flexibility in choosing exposure parameters (e.g., frequency, modulation schemes, etc.) as well as permit optimal handling of the cell and tissue samples in the absence of discernible heating.

To work further toward developing effective applications of pulsed, high intensity RF/MW electromagnetic fields, we expanded collaborative efforts with researchers Thomas Vernier and Martin Gundersen at USC. Given that our long term objective is to develop directed energy non-lethal weapons, the simultaneous research focus on identifying and elucidating bioeffects due to both unipolar pulses and RF/MW pulses of high electric field intensity will greatly accelerate the goal of coming up with practical outcomes for our research.

Specific Projects and Outcomes

1) Identifying non-thermal RF/MW effects (1 to 6 GHz frequency range) on catecholamine release: The free space exposure setup for exposing chromaffin cells to MW fields in the 1 to 6 GHz frequency range (published in Yoon et al., 2006) was modified to achieve better performance and thus enable us to conduct well-controlled biological experiments. Some of the modifications that were made included improvements to the following elements of the perfusion/on-line electrochemical detection system: cell perfusion apparatus (CPA), pressure stabilization, drug-stimulus injection, temperature control, and automation. A schematic of the exposure system is shown in Figure 1 (Appendix). It should be noted that the improvements to the exposure system also allowed higher electric fields with good homogeneity to be generated where the chromaffin cells are located (Table 1, Appendix and Figure 2, Appendix).

Briefly, the exposure system consists of a CPA inside which chromaffin cells are immobilized on a glass fiber filter (GFF) of diameter 10 mm. The cells are continuously superfused at a rate of 1.0 ml/min with a balanced salt solution (BSS) maintained at $36.5 \pm 0.2^\circ\text{C}$. The temperature of the BSS entering and exiting the CPA is continuously monitored in the inlet and outlet tubing with non-perturbing fluoroptic temperature probes placed as close as physically possible to the GFF where the cells are immobilized. The CPA is mounted vertically within a mini anechoic chamber and the cells exposed to MW fields in the frequency range 1 - 6 GHz

by positioning the CPA in the near field of a high power broadband horn antenna. Catecholamine release is monitored by an electrochemical detector placed in-line with the CPA outlet tubing. Figure 3 (Appendix) shows results from a typical control experiment (i.e., no MW exposure) in which catecholamine release was stimulated by repetitive applications of a submaximal concentration of the nicotinic receptor agonist 1,1-dimethyl-4-piperazinium (DMPP). With successive applications of DMPP, the overall areas under the peaks and hence the amount of catecholamine released gradually decreases in a manner that follows an exponential decay model, a finding which agrees with results described by other researchers who similarly use an on-line electrochemical detection system to monitor the release of catecholamine during cell perfusion. The last peak that is larger represents the response of the cells to twice the amount of DMPP.

A series of studies were carried out delivering continuous wave and pulsed MW fields at either fixed frequencies or using the novel exposure paradigm of pulsed frequency sweeps (PFS; each sweep spanning one GHz), with much data accumulated. When no effects of MW exposure were observed, the profile of catecholamine release in response to DMPP exhibited the same exponential decay pattern as the corresponding control experiment. In contrast, possible MW-induced bioeffects manifested themselves as either increases or decreases in DMPP-stimulated catecholamine release during and/or after MW exposure that were outside the 95% prediction band for the exponential decay pattern of the corresponding control experiment. Figure 4a (Appendix) provides an example of the results of an experiment in which chromaffin cells were exposed to PFS 5-6 GHz fields and effects on DMPP-stimulated catecholamine release observed that were considered to represent bioeffects. Figure 4b shows that both temperature and pressure, which can influence both the release of catecholamine from the cells and the output of the electrochemical detector, were maintained constant during the exposures.

Our analysis of the results obtained so far show that of several PFS exposure experiments carried out in the 1 to 6 GHz frequency range with a variety of parameters, a frequency sweep range from 5 to 6 GHz using a pulse width of 100 ms (Figure 5, Appendix) showed significant bioeffects most often. These results are being investigated further while the data already obtained are being prepared for submission as a manuscript. This work will also serve as partial fulfillment of the requirement for a doctoral degree to be awarded to Jihwan Yoon.

2. Novel RF/MW exposure setup for real-time monitoring of effects on chromaffin cells: A novel exposure system that can deliver high electric field RF/MW pulse modulated radiation, broadband Gaussian pulses or RF/MW modulated Gaussian pulses with frequency spectrum centered in the band 0.75 – 6 GHz has been fabricated for use with an inverted microscope for real-time fluorescence imaging of effects on chromaffin cells, such as changes in intracellular calcium. A photograph of the setup is presented in Figure 6 (Appendix). Briefly, chromaffin cells are immobilized on collagen-coated indium tin oxide (ITO) cover slips, which comprise the bottom of a specially designed circular cell perfusion chamber (Figure 6, Appendix). Cells are loaded with the fluorescent calcium indicator dye, Calcium Green-1 and the cell chamber attached to the exposure device in which RF/MW fields are delivered to the cells by means of a carefully designed coaxial applicator having a highly tapered inner conductor (Figure 7, Appendix). The entire setup is mounted on the stage of an epifluorescence microscope, and images of the cells are captured before, during and after exposure. Cells are continuously perfused throughout an experiment with BSS maintained at $36.5 \pm 0.2^{\circ}\text{C}$ and DMPP injected at varying intervals for assessing effects on both basal intracellular calcium level and nicotinic receptor mediated increases in intracellular calcium level. Figure 8 (Appendix) shows an example of how intracellular calcium increases after multiple applications of DMPP to the cells. Experiments are now underway in which we are first carrying out the appropriate control experiments to ensure proper temperature maintenance during the variety of exposure conditions to be employed.

3) Identifying non-thermal RF/MW effects on skeletal muscle contraction: Experiments have been underway using a waveguide-based exposure system for the 750 to 1000 MHz frequency range that incorporates an organ bath (published in Lambrecht et al., 2006). Photographs of the entire setup with all recent modifications since that publication are shown in Figure 9 (Appendix).

The experimental strategy is as follows. The fast twitch skeletal muscle, *flexor digitorum brevis* from the hind foot of the mouse, is stimulated to contract electrically at frequencies ranging from 1 to 100 Hz (Figure 10, Appendix) in the absence and then presence of RF fields. Initial experiments carried out using pulsed fields that employed different pulse widths and pulse repetition rates (PRR) at several fixed frequencies ranging from 750 to 1000 MHz showed no effects on contractile force. A second strategy recently employed was to deliver pulsed RF fields during frequency sweeps spanning a 100 MHz range. In essence, a similar PFS strategy as that for looking at MW effects on catecholamine release from chromaffin cells was employed here. An example of a specific PFS exposure paradigm that was used is given in Figure 11 (Appendix) for a frequency sweep from 750 to 850 MHz, then in the opposite direction from 850 to 750 MHz, the goal being to deliver pulsed fields with continuous variation in frequency and input power. To date, no effects on contractile force have been observed at the pulse widths and PRR used so far. Experiments aimed at finishing this analysis using different pulse widths and PRR will continue and will include exposure protocols in which the length of time for the frequency sweep is reduced to produce changes electric field intensity in shorter periods of time period.

4) Effect of nanosecond pulses on chromaffin cells: Studies assessing the effects of nanosecond duration, high intensity electric pulses on chromaffin cells constitute a recent and productive collaboration with Tom Vernier and Martin Gundersen at USC. The research got underway when chromaffin cells were sent to Tom Vernier and a study was conducted in his laboratory to examine, via fluorescence microscopy, how intracellular calcium level is affected by the application of one or more 4 nanosecond pulses. As reported in Vernier et al. (2008), a single 4 nanosecond pulse at a field intensity of 5 MV/m elicits a rapid and transient increase in intracellular calcium that is due to calcium influx across the plasma membrane. Recently the group at USC fabricated a nanosecond pulser and a pulser interface board for us so that these studies can continue at UNR. They also provided us with the method to fabricate the microelectrode chambers needed for carrying out these experiments. These studies are now underway and Figure 12 (Appendix) is a photograph of the setup.

Because calcium entry into chromaffin cells is the primary trigger for stimulating the release of catecholamines, a critical question is whether the single burst of calcium influx elicited by a single 4 nanosecond pulse is sufficient in magnitude to be of physiological significance, that is, to result in the release of catecholamines. To address this question, USC provided us with a magnetic compression diode-opening switch cuvette pulse generator and catecholamine release is being assessed as follows: 40,000 cells in 80 μ l of a balanced salt solution (BSS) are placed into standard electroporation cuvettes (1 mm electrode spacing) that serve as the exposure chambers; cell exposure is carried out at room temperature. Two minutes after pulse delivery, the cuvettes are cooled on ice and the cells pelleted in the cuvettes by centrifugation. Aliquots of the supernatant fraction are analyzed by high performance liquid chromatography coupled with electrochemical detection to assess the amount of the catecholamines norepinephrine and epinephrine released from the cells. Figure 13 (Appendix) is a photograph of the setup and Table 2 (Appendix) presents the results of preliminary studies that show a stimulation of norepinephrine and epinephrine release that is similar in magnitude to that elicited by a submaximal concentration of DMPP. Similar to the findings observed by fluorescence imaging of intracellular calcium level, nanosecond pulse-stimulated catecholamine release is dependent on extracellular calcium (Table 3, Appendix). As determined by addition of the dye YO-PRO-1 to the cells during pulsing, detectable uptake of the dye does not occur in response to a single nanoelectropulse (Figure 14, Appendix), suggesting the absence of plasma membrane electroporation and hence any overt deleterious effects.

Ongoing work/future directions.

Efforts will be directed at understanding the basis of the apparent effects of PFS 1-6 GHz fields on catecholamine release from chromaffin cells. While the data so far indicate that the effects occur in the absence of overt heating, micro heating may still be occurring. This will have to be investigated. Adding to our ability to understand the mechanism of any apparent bioeffects, including the possibility of micro heating, will be the use of the new RF/MW exposure setup that will allow us to conduct experiments and observe responses of the cells in real time.

With respect to studies investigating the effects of RF fields on skeletal muscle contraction, we will not only be completing experiments that monitor force production but also do a follow-up series of studies that monitor the rate of force production, as this could be another way that the muscle can be affected.

Studies examining the effect of nanosecond high electric field intensity on chromaffin cells will proceed. Since heating is not an issue, the goal will be to work toward identifying the mechanism underlying the influx of calcium and subsequent release of catecholamine.

APPENDIX

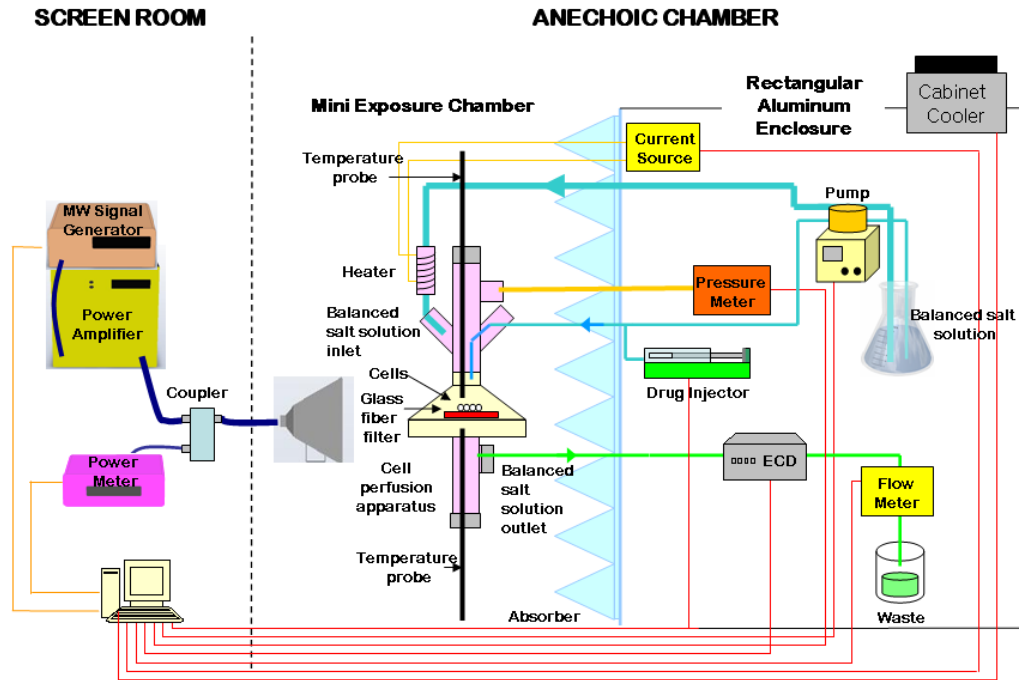


Figure 1. Schematic of the modified free space MW exposure system showing the experimental arrangements inside the anechoic chamber and adjacent screen room.

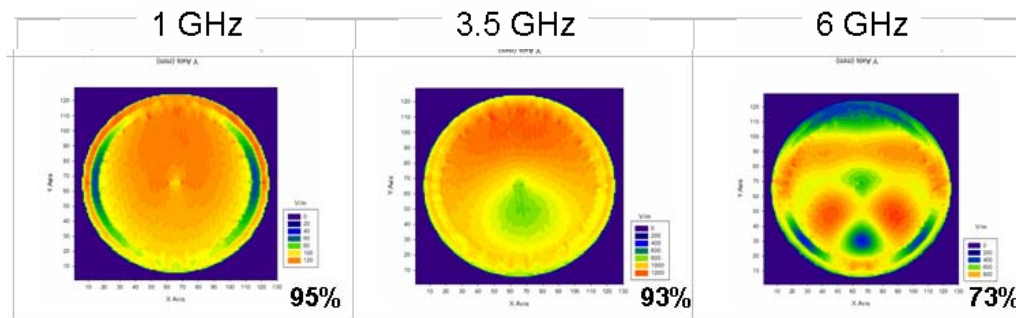
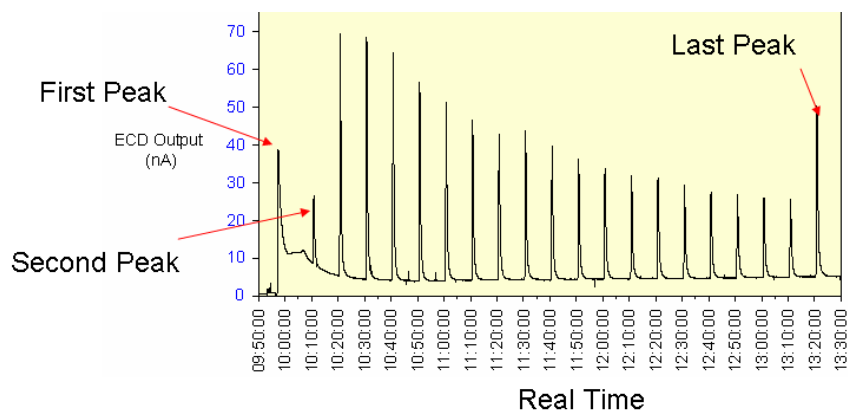


Figure 2. Electric field distributions in the near field at 1, 3.5 and 6 GHz, where % refers to the area of the GFF homogeneous to within 30%.

a)



b)

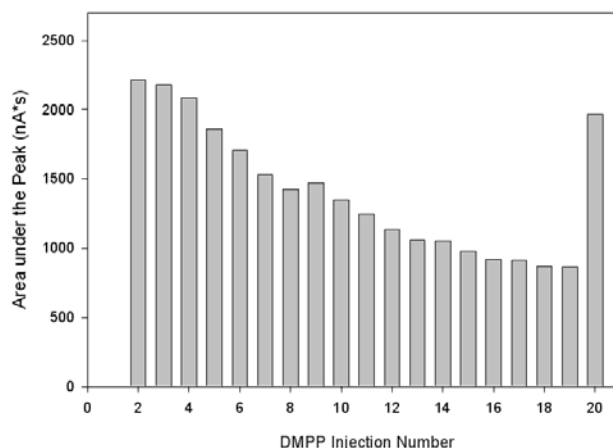
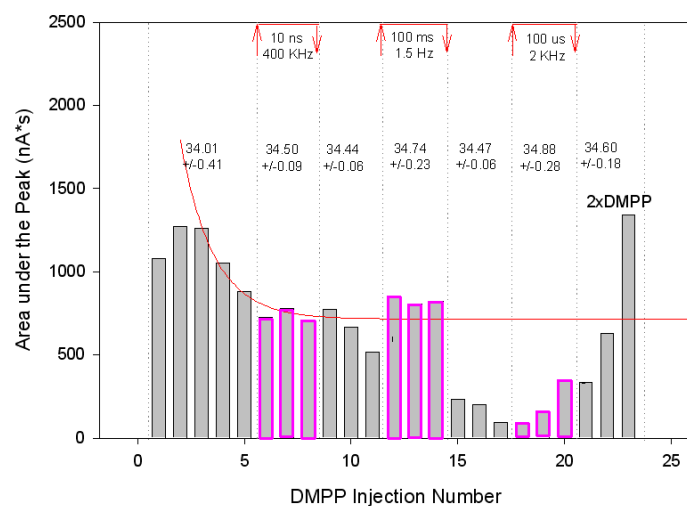


Figure 3. Catecholamine release profile for a typical control experiment in which DMPP was injected every ten minutes. a) electrochemical detector (ECD) output. The first peak represents catecholamine release stimulated in response to the sudden fluctuation in pressure and flow rate while the CPA containing the chromaffin cells is being connected to the flow-through system and the second peak represents the first response to DMPP that is smaller than successive peaks because the injection syringe has not yet overcome the pressure built up in the main BSS inlet tubing. The last peak represents catecholamine release in response to twice the amount of injected DMPP. b) Bar graph showing the calculated area under the catecholamine peaks. Not shown are the areas for the first two peaks.

a)



b)

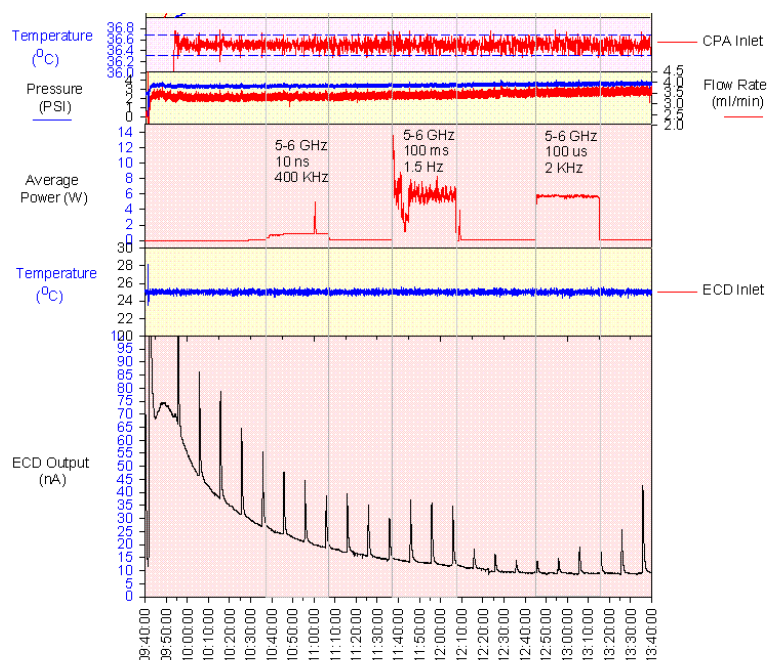


Figure 4. Bar graph (a) showing the calculated area under the catecholamine peaks for cells exposed to 5-6 GHz PFS with a sweep time of 500 ms, pulse widths of either 10 ns, 100 ms or 100 us, and a PRR rate of 1-2 Hz. The orange line represents the predicted trend line for the exponential decay of the catecholamine peaks. (b) Continuous monitoring of temperature, pressure and electrochemical detector (ECD) output during the MW exposure experiment shown in a). When the MW field was applied, average power was also monitored.

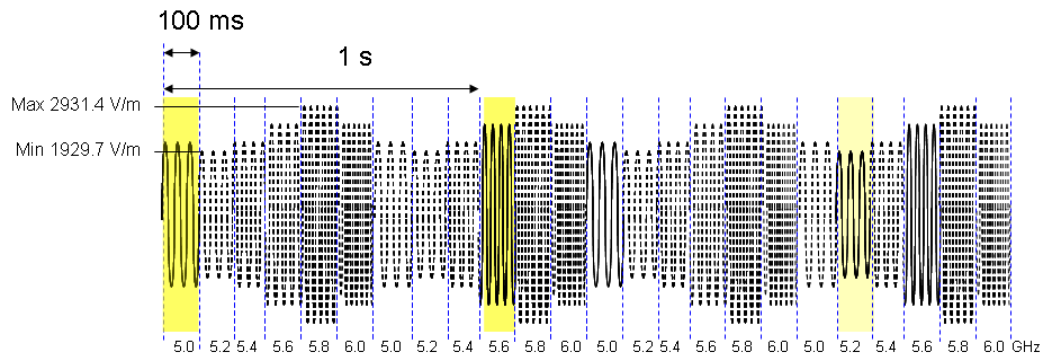


Figure 5. Illustration of the 5-6 GHz PFS with sweep time of 500 ms, pulse width of 100 ms and the repetition rate of 1 Hz at the location of the cells. The magnitudes of the wave at different frequencies vary since the gain of the amplifier, the gain of the broadband horn antenna and the loss of the power cable vary different at frequencies. The magnitudes were found using XFDTD results and the measured cable loss.

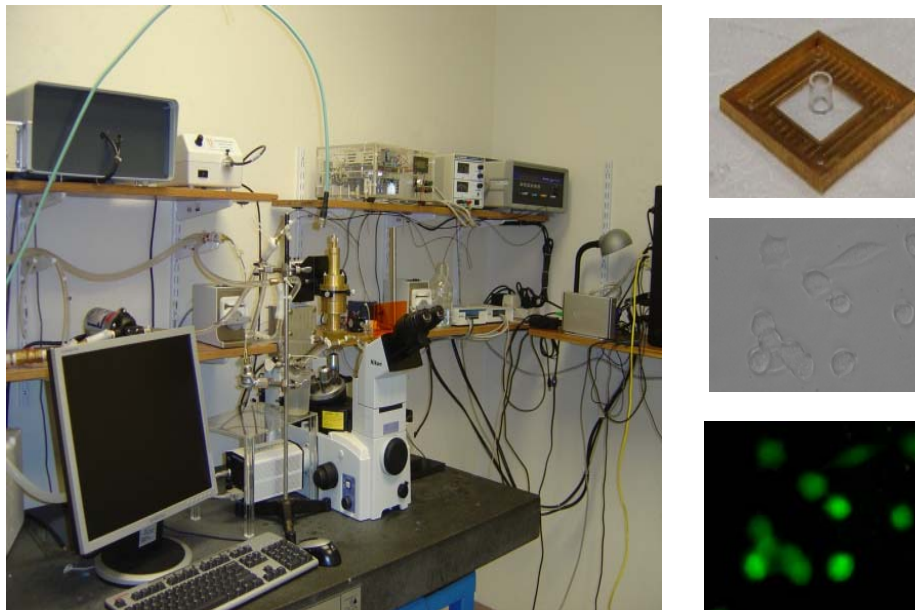


Figure 6. Photograph of the exposure device mounted on the inverted microscope (left) and of the cell chamber (top right). Bright field (middle right) and corresponding fluorescent field (bottom right) of chromaffin cells attached to the bottom of the cell chamber and loaded with the calcium indicator dye Calcium Green-1.

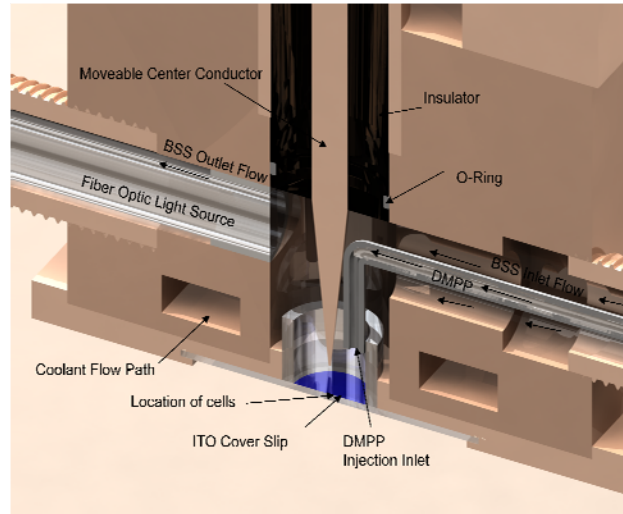


Figure 7. SolidWorks rendering of the exposure device cross section.

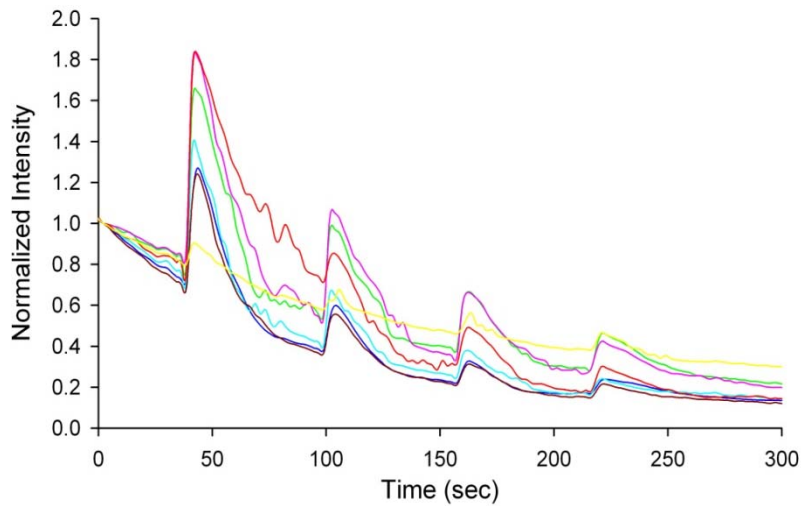


Figure 8. Effect of multiple applications of DMPP on intracellular calcium level in chromaffin cells. The flow rate of the BSS was 2 ml/min and DMPP (0.1 ml of 1 mM in BSS) was injected into the cell chamber at four different times. There were seven cells in the visual field and all showed a response to DMPP. The diminished response over time was due to photobleaching of the dye.

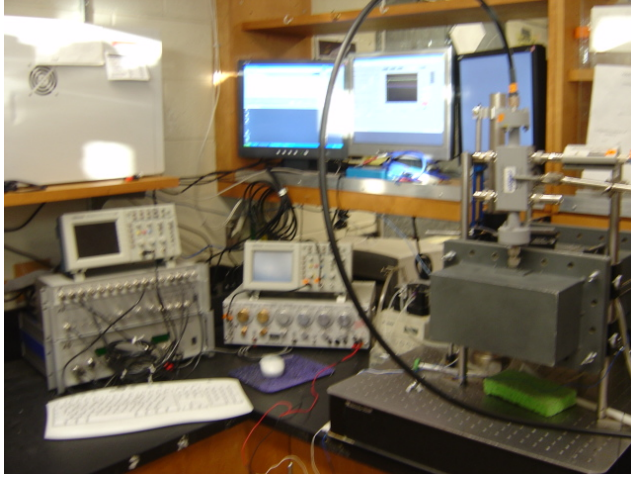


Figure 9. Photograph of the waveguide-based exposure system for assessing RF effects on skeletal muscle contraction (left) and of the RF generating equipment (right).

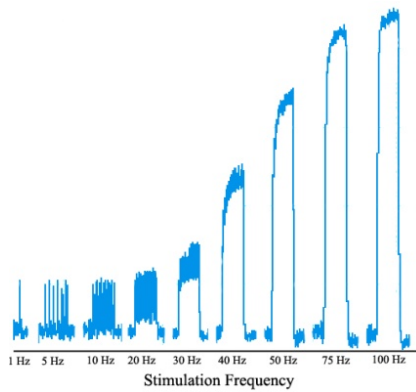


Figure 10. Contractile force generated as a function of electric stimulation frequency. Low frequency electrical stimulations produce single twitches that fuse to produce much greater force (tetanic force) as the stimulation frequency increases.

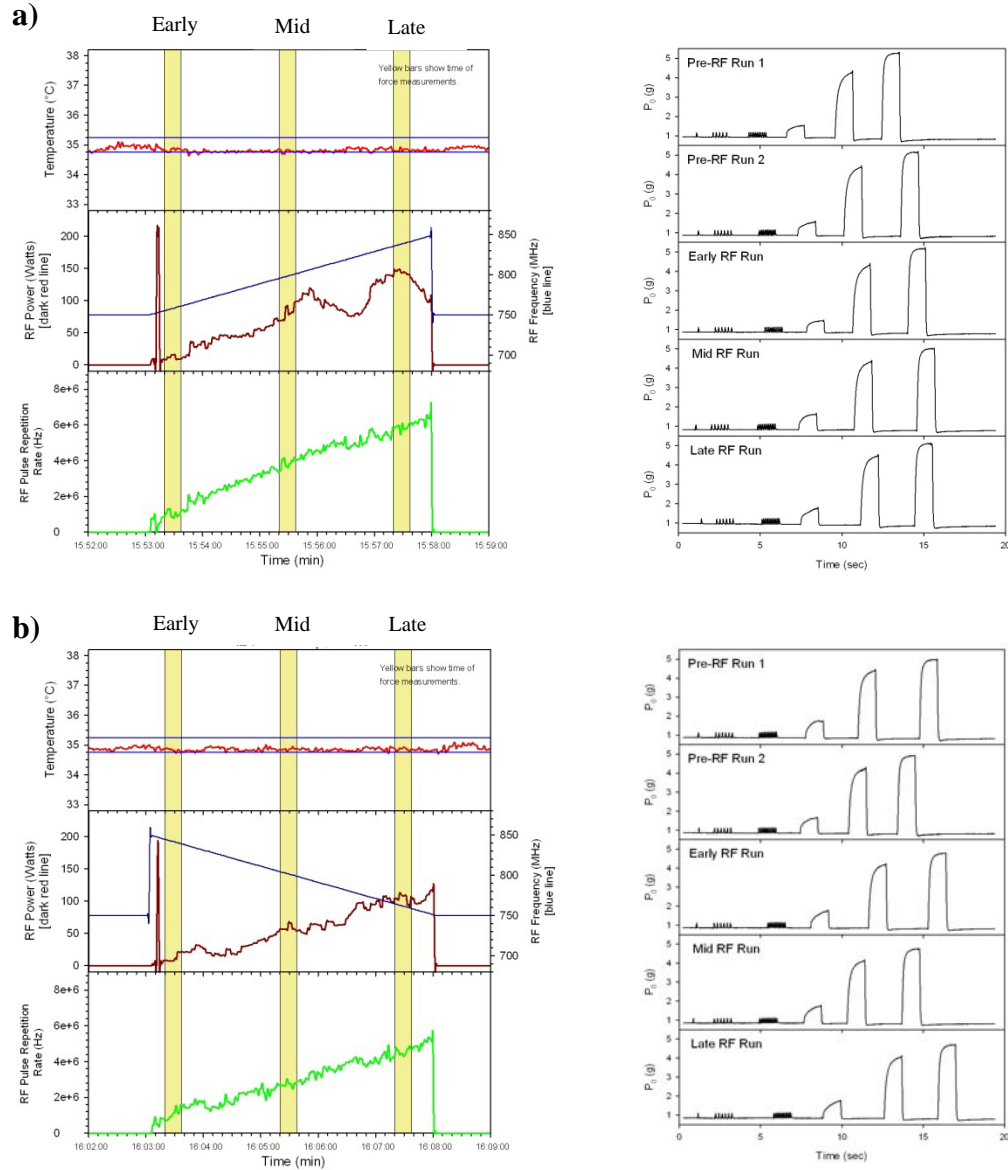


Figure 11. Representative experiment in which skeletal muscle contraction was assessed during exposure to PFS fields at 750-850 MHz. a) The yellow vertical bars in the graph at the left indicate the point in time at which a series of electrical stimulations of 1, 5, 10, 30, 60 and 100 Hz were delivered to the muscle. The panel on the right shows the contractile traces from those stimulations (Early, Mid, and Late RF Runs) as well as the contractile traces obtained before field application. The graph at the left also shows that the applied PFS field employed a frequency sweep going from 750 to 850 MHz, a PRR in the range 1-10 MHz (pulse width of 60 ns), and a frequency sweep time of 5 minutes. Temperature was also monitored (top panel) and showed relatively constant values during the exposure. b) Same as for a) except that the frequency sweep was from 850 to 750 MHz.

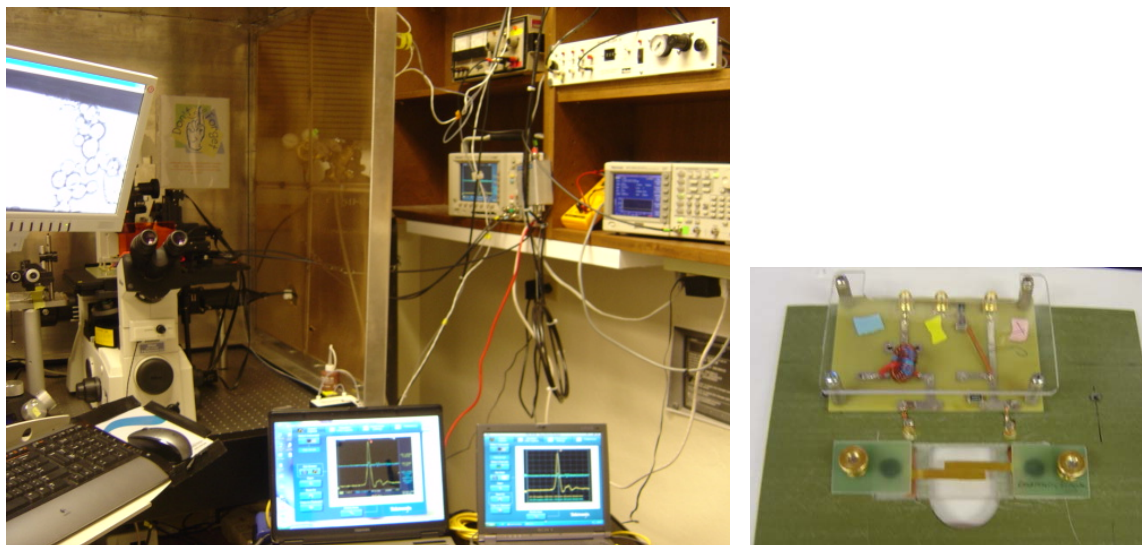


Figure 12. Photograph of the setup for observing effects of nanosecond pulses via fluorescence imaging (left). Exposures are carried out at room temperature within a microelectrode chamber (photograph at right) mounted on the stage of a Nikon TE 2000 epifluorescence microscope, and intracellular calcium level monitored in real-time in cells loaded with the calcium indicator dye, Calcium Green-1.

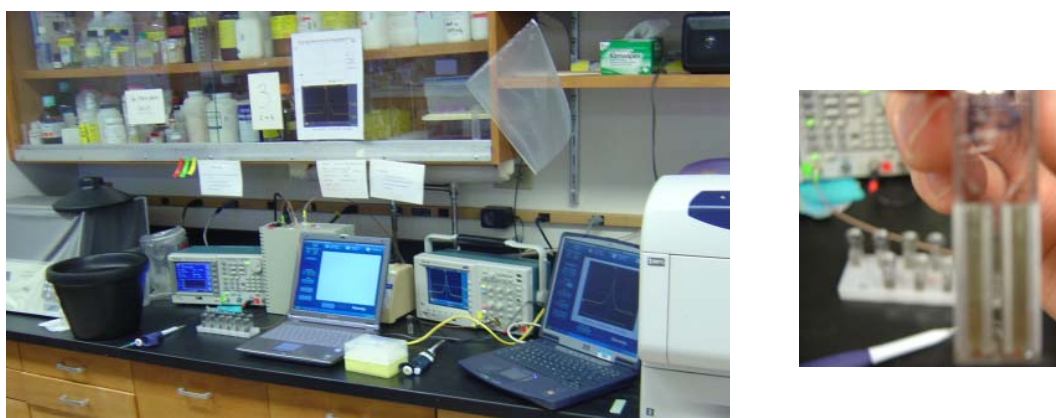


Figure 13. Photograph of the setup for assessing the effect of nanosecond electric pulses of high field intensity on catecholamine release from chromaffin cells (left) and of an electroporation cuvette that is used as an exposure chamber (right).

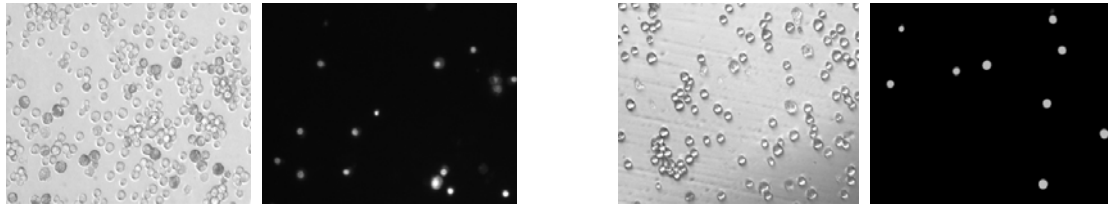


Figure 14. Determination of plasma membrane integrity for cells exposed to a single 4 nanosecond, 5 MV/m pulse in the presence of the non-permeable dye YO-PRO-1. Left: bright field and fluorescent field, respectively, for cells not pulsed; Right: bright field and fluorescence field, respectively, for pulsed cells. Note that there is no increase in YO-PRO-1 influx (i.e., number of cells that fluoresce) after the application of a single pulse.

Table 1. Adjusted electric field magnitudes based on the maximum power and the cable loss. Rectangle in red indicates the values for the current exposure setup that uses a smaller CPA than the original system described in Yoon et al., 2006.

Frequency (GHz)	Original CPA						Smaller CPA		
	Far Field			Near Field			Near Field		
	Mean (V/m)	Max (V/m)	Min (V/m)	Mean (V/m)	Max (V/m)	Min (V/m)	Mean (V/m)	Max (V/m)	Min (V/m)
1	10,3	13,6	4,6	105,2	120,3	674,7	966,8	1082,7	677,5
3,5	300	393,4	93,1	976,2	1166,3	886,9	1302,1	1415,5	1069,4
6	110,2	206,1	26,3	674,7	392	392	2393,4	2790,0	1874,5

Table 2. Effect of a single 4 nanosecond pulse alone or with a submaximal concentration of DMPP on norepinephrine (NE) and epinephrine (EPI) release.

STIMULUS	NE STORES RELEASED (%)	EPI STORES RELEASED (%)
	(mean \pm SEM)	(mean \pm SEM)
DMPP (n = 15)	5.31 \pm 0.40	3.42 \pm 0.30
Pulse (n = 15)	4.37 \pm 0.51	3.64 \pm 0.51
	(mean \pm SD)	(mean \pm SD)
DMPP (n = 2)	5.58 \pm 0.62	2.22 \pm 0.49
Pulse (n = 2)	5.38 \pm 1.01	3.35 \pm 0.87
DMPP + Pulse (n = 2)	6.33 \pm 0.14	3.43 \pm 0.95

Table 3. Effect of extracellular Ca^{2+} on norepinephrine (NE) and epinephrine (EPI) release stimulated by DMPP or a 4 nanosecond pulse.

STIMULUS	NE STORES RELEASED	EPI STORES RELEASED
	(%)	(%)
	(mean \pm SD)	(mean \pm SD)
DMPP (+ Ca^{2+})	5.65 \pm 2.45	3.20 \pm 1.41
(- Ca^{2+})	None	None
Pulse (+ Ca^{2+})	3.58 \pm 1.21	2.73 \pm 0.84
(- Ca^{2+})	None	None